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Endotoxin and (1→3)-β-D-Glucan Contamination in Electronic Cigarette Products Sold in the United States

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Background: Cigarette smoke contains microbes and microbial toxins, such as endotoxin and (1 → 3)-β-D-glucan, that may have adverse respiratory effects. To our knowledge, the potential for contamination of electronic cigarette (EC) products sold in the United States has not been investigated.

Objectives: We aimed to determine whether popular cartridge and e-liquid EC products were contaminated with endotoxin or glucan and to examine differences according to the type and flavor of products.

Methods: We selected 37 cartridges and 38 e-liquid products with the highest nicotine content from the ten top-selling U.S. brands. Flavors were classified into four groups: tobacco, menthol, fruit, and other. Endotoxin and glucan were measured using an endotoxin-specific kinetic turbidimetric assay and a Glucatell® Kinetic Assay (Associates of Cape Cod, Inc.), respectively.

Results: Endotoxin concentrations were over the limit of detection (LOD) in 17 of 75 products tested (23%), and glucan concentrations were greater than LOD in 61 of 75 products (81%). After adjusting for brand and flavor, the mean glucan concentration was 3.2 times higher [95% confidence interval (CI): −0.1, 18.4] in cartridge vs. e-liquid samples. After adjusting for brand and type of product, glucan concentrations in tobacco- and menthol-flavored ECs were 10.4 (95% CI: 1.8, 44.9) and 3.5 (95% CI: 0.1, 17.3) times higher than concentrations found in fruit-flavored products.

Conclusions: EC products may be contaminated with microbial toxins. Further studies with large representative samples of products are needed to confirm our findings, identify sources and routes of contamination, and evaluate health effects associated with the use of contaminated products. https://doi.org/10.1289/EHP3469

Introduction

The use of e-cigarettes (ECs), also called electronic nicotine delivery systems (ENDS), has increased substantially, with sales in the United States reaching an estimated $3.3 billion in 2015, a 32% increase from $2.5 billion in 2014 (Marynak et al. 2017). Based on the U.S. National Youth Tobacco Survey (NYTS), use among U.S. teens increased 1.5% (220,000 students) to 20.8% (3.05 million students) among high school students, and 0.6% (60,000 students) to 4.9% (570,000 students) among middle school students between 2011 and 2018 (Cullen et al. 2018), and EC products were the most commonly used by U.S. teens in 2017, exceeding reported use of cigarettes, chewing tobacco, cigars, and hookahs (Wang et al. 2018). Furthermore, in a nationally representative sample of U.S. teens (12–17 years of age) who never smoked a conventional cigarette before enrollment, those who reported ever using EC at baseline were 3.5 times [95% confidence interval (CI): 2.5, 4.9] more likely than never-EC users to have smoked at least one cigarette a year later (Watkins et al. 2018).

Although the use of ECs continues to climb, data on exposures and potential human health effects are lacking. Investigations have focused on chemical content, such as nicotine, tobacco-specific nitrosamines, carbonyl compounds, aldehydes, fine particulate matter, metals, volatile organic compounds (VOCs), flavorings, and other additives, which have been found in various EC matrices, including refill solutions, cartridges, aerosols, and environmental emissions, and in aqueous solution (Allen et al. 2016; Bekki et al. 2014; Cheng 2014; Farsalinos et al. 2014, 2015; Fernández et al. 2015; Geiss et al. 2015; Goniewicz et al. 2014a, 2014b; Ingebrethsen et al. 2012; Jensen et al. 2015; Klager et al. 2017; Lee et al. 2017; Melstrom et al. 2017; Orr 2014; Pankow et al. 2017; Park et al. 2019; Schober et al. 2014; Uchiyama et al. 2013; Williams et al. 2013). However, to our knowledge, no data are available on the contamination of EC products with microbes or microbial toxins.

Microbial agents such as endotoxin [or lipopolysaccharide (LPS)], part of the outer membrane of Gram-negative bacteria (Bos and Tommassen 2004), and (1 → 3)-β-D-glucan, a fungal cell wall constituent, have been associated with adverse respiratory health outcomes (Adhikari et al. 2011; Douwes 2005; Maheswaran et al. 2014; Pauly and Paszkiewicz 2011).

Endotoxin is ubiquitous in the environment and is present at higher concentrations in tobacco smoke than in smoke-free indoor air (Larsson et al. 2004; Szponar et al. 2012). Endotoxin also has been identified in occupational settings, such as cotton-textile workplaces (Lai et al. 2012, 2015), agricultural environments (e.g., livestock, dairy) (Donham 2010; Basinas et al. 2015), and waste-incineration facilities (Park et al. 2011), as well as in residential environments (Carnes et al. 2017; Holst et al. 2015; Lee et al. 2018; Yoda et al. 2017). Endotoxin exposure causes emphysematous changes (Brass et al. 2008) and airway remodeling (Brass et al. 2003) in experimental animals. In humans, occupational endotoxin exposure has been associated with the development of airflow obstruction, respiratory symptoms, reduced lung function, and current atopic and nonatopic asthma (Carnes et al. 2017; Castellan et al. 1987; Lai et al. 2012, 2015). Household endotoxin exposure has been also associated with increased peripheral leucocyte count, a biomarker of inflammation (Fessler et al. 2017), and increased asthma prevalence in the National Health and

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Nutrition Examination Survey (NHANES) 2005–2006 (Thorne et al. 2015).

(1 → 3)-β-D-glucan is a polymer of glucose that is present in the cell walls of most fungi, plants, certain bacteria, and algae (Douwes 2005). Airborne (1 → 3)-β-D-glucan has been used as a surrogate to estimate the human exposure to fungi in indoor environments (Adhikari et al. 2011; Douwes et al. 2006; Iossifova et al. 2008; Schram-Bijkerk et al. 2005). (1 → 3)-β-D-glucan in house dust has been associated with a greater annual decline in forced expiratory volume in 1 s (FEV_1) among residents of a rowhouse (Thorn and Rylander 1998) and persistent atopic asthma and the onset of bronchial hyperresponsiveness (BHR) in adolescent children (Maheswaran et al. 2014). These bacterial and fungal components are believed to contribute, in part, to lung inflammation in smokers (Pauly and Paszkiewicz 2011).

Although contamination of e-liquid (also called e-juice) with microbes or microbial toxins is possible, to our knowledge no study of microbial contamination of EC products has been published. Therefore, as a first step toward assessing potential hazards to EC users, we assayed samples of EC cartridges and e-liquids sold by the top 10 retail brands in the United States for endotoxin and glucan contamination.

Methods

Selection of EC Cartridges and E-Liquids

The total number of products tested was determined by the availability of funds to cover the cost of the assays. Specific EC products included in our study sample (total $n = 75$) were selected based on the following criteria. First, we identified the 10 top-selling EC product brands in the United States during 2013 (hereafter referred to as Brands A–J) based on Nielsen Scantrak Data, which reflect representative sales estimates that were derived from information from in-store scanners and field audits of retail outlets without scanners (Giovenco et al. 2015; Herzog et al. 2014). Next, we ranked all cartridge products (first generation, also known as cigalikes) and all e-liquid products (refillable e-liquid bottles) sold by the top 10 brands. This selection included all the flavors, except the mixed flavor, as indicated on product labels (Brand I, 8 samples). If labels listed more than one available nicotine content amount for each flavor by brand and type, we selected the product with the highest nicotine content as indicated on product labels. Finally, the total 75 samples included 37 cartridges and 38 e-liquids samples (Figure S1). All selected products were available online and purchased from the EC company websites (9 company websites, 69 samples), except the products from one brand (Brand G). We purchased the products from that brand (Brand G, 6 samples) from the convenience store located near the Harvard T.H. Chan School of Public Health based on convenience and costs. After purchase, products were stored in a dark room at room temperature about 20 to 22 degrees celsius in their original packages until they were shipped to the laboratory where all sample preparation and assays were performed (Associates of Cape Cod, Inc., East Falmouth, Massachusetts).

Quantification of Microbial Markers

Sample preparation. All samples were extracted in a laminar flow hood using aseptic techniques.

Cartridges. EC cartridges, which are cylinder-like chambers, contain fibrous pads that absorb the e-liquid and act as a wick to deliver the liquid to the atomizer. Depyrogenated forceps were used to remove the pad from each cartridge and squeeze the liquid from it. Liquid from three cartridges of the same product from the same package was pooled in a depyrogenated beaker and transferred to depyrogenated glass tubes for retention and testing. The samples were diluted with LRW in serial dilution and tested, in duplicate, to find minimum noninterfering dilutions.

E-Liquids. E-liquid samples were not pooled, and a small portion of e-liquid was taken from an e-liquid bottle. In the same manner as the cartridge samples were taken, the e-liquid samples were diluted and tested to find minimum noninterfering dilutions.

Sample assays. **Endotoxin.** Endotoxin was measured using an endotoxin-specific kinetic turbidimetric in a *Limulus* amebocyte lysate (LAL) assay. Samples were tested in duplicate with *Limulus* reagent water (LRW) in serial dilutions ranging from 1:20 to 1:320,000 to find the minimum noninterfering dilution. The limits of detection (LODs) for the endotoxin assays ranged from 0.1 to 1.6 endotoxin units (EU)/mL. All absolute values for the correlation coefficients for the calibration curves using Control Standard Endotoxin (Associates of Cape Cod, Inc.) were ≥0.980. Negative controls (LRW) for each batch were lower than lowest standard (0.001 EU/mL or 0.002 EU/mL), and recovery of product positive controls (0.008 EU/mL; Control Standard Endotoxin) ranged 50% to 200% for endotoxin. These assay parameters, including correlation coefficients, negative controls, and positive product controls, met the U.S. Pharmacopeia (USP) requirement for the endotoxin assay (USP 2016).

(1 → 3)-β-D-glucan. (1 → 3)-β-D-glucan was measured using a Glucatell® Kinetic Assay (Associates of Cape Cod, Inc.). Samples were tested in duplicate with LRW in serial dilutions from 1:20 to 1:320,000 to find the minimum noninterfering dilution. All absolute values for the correlation coefficients for the calibration curves using (1 → 3)-β-D-glucan standard (*Pachyman*, Associates of Cape Cod, Inc.) were ≥0.980. LODs ranged from 0.0125 to 0.2 ng/mL. LRW controls for each batch were lower than lowest standard (3.125 pg/mL or 6.25 pg/mL). USP requirements had not been set for the glucan assay at the time our study was performed.

Replicate samples. We used simple random samplings (SAS® PROC SURVEYSELECT; version 9.4, SAS Institute Inc.) to select 4 cartridge samples out of 37 cartridge products and 4 e-liquid samples out of 38 e-liquid products, respectively (more than 10% of total sample size) for replicate assays (Table S1). Samplings were without replacement, and the selection probability for each cartridge and e-liquid equals 0.108 and 0.105, respectively. For cartridge samples, we pooled liquid from three cartridges for the replicate sample from the same package for each product selected (i.e., testing a primary sample and a replicate sample from the same package). For e-liquid samples, we assayed liquid from the same bottle (i.e., testing a primary sample and a replicate sample from the same bottle). For glucan, the coefficient of variance (CV) for each of the 8 pairs of replicate samples ranged from 1.3% to 22.4% (Table S1). Endotoxin concentrations were less than LOD in all samples tested.

Statistical Analysis

Data were analyzed using the SAS Statistical Package (version 9.4; SAS Institute Inc.). Values below the LOD for endotoxin or glucan were imputed as the LOD/2 (Allen et al. 2016). Descriptive statistics include the mean, median, and range of microbial markers, according to product type (cartridge or e-liquid), flavor (4 groups), and brand. Product flavors were categorized as tobacco, menthol, fruit, or other (Table 1), based on product names and descriptions provided on distributor websites, consistent with Nielsen classifications (Giovenco et al. 2015). The fruit category included any flavor that referenced a fruit or fruit-like product (e.g., peach, strawberry); the category titled other included miscellaneous flavors, such as vanilla, chocolate, and

Table 1. EC Flavor categories according Nielsen classification.

Flavor type	Flavors in this group
Tobacco	Classic tobacco, platinum label tobacco, bold tobacco, gold tobacco, regular, traditional tobacco, tobacco-bold, original, tobacco, traditional, classic, American blend (tobacco), pro-platinum label tobacco
Menthol	Magnificent menthol, platinum label menthol, menthol, menthol-bold, cool ice blend (menthol), pro-platinum label menthol
Fruit	Cherry crush, peach schnapps, pomegranate, berry, acai berry, strawberry, peach, Washington red, ocean mist (melon), grape, mango, apple, berry, pineapple, watermelon, menthol citrus, citrus crush, Havana, tropical fruit, blue + black berry, peach tea
Others	Java jolt, vivid vanilla, piña colada, mint, cream, chai, vanilla, fusion, winter mint, java (coffee), vanilla bean

Note: Giovenco et al. 2015; Herzog et al. 2014.

coffee. We also derived Spearman correlations between $\log_{10}$-transformed endotoxin and glucan concentrations in each sample. We used linear regressions to estimate differences in $\log_{10}$-transformed endotoxin and glucan concentrations, according to product type (cartridge vs. e-liquid) and flavor (tobacco, menthol, or other vs. fruit), before and after adjusting for brand (modeled using indicator terms) and either flavor or type, as appropriate. Model estimates were converted to percent differences as $(10^{\beta} - 1) \times 100\%$, where β is the estimated regression coefficient.

Results

Among the 75 EC products tested, endotoxin concentrations were greater than LOD in 17 products (23%) and glucan concentrations were greater than LOD in 61 products (81%) (Table 2). One product (a fruit-flavored e-liquid, brand F, Table S2) was greater than LOD for endotoxin but less than LOD for glucan; otherwise, all products that were greater than LOD for endotoxin were also greater than LOD for glucan. Endotoxin concentrations were greater than LOD in 12 of 37 cartridge products, and in 4 of 16, 1 of 15, 7 of 29, and 5 of 15 tobacco-, menthol-, fruit-, and other-flavored products, respectively. Glucan concentrations were greater than LOD in all cartridge products and in 16 of 16 tobacco-flavored products, 13 of 15 menthol-flavored products, 19 of 29 fruit-flavored products, and 13 of 15 other-flavored, respectively. When evaluated by brand, endotoxin concentrations were greater than LOD in at least one product from 7 of the 10 brands, whereas glucan concentrations were greater than LOD in every product tested for 8 brands, and in 3 of 7 and 8 of 18 products from Brands F and I, respectively. Both microbial contaminants were less than LOD in 13 products (17%), including 3 of 7 products from Brand F, and 10 of 18 products from Brand I. Information on the brand, type, flavor, and endotoxin and glucan concentrations of each of the 75 samples tested is provided in Table S2.

After substituting values less than LOD with LOD/2, geometric mean concentrations (± GSD) of endotoxin and glucan in all tested EC products were 0.14 EU/mL (± 3.18, range 0.05–1.64 EU/mL) and 1.01 g/mL (± 18.95, range 0.01–1,450.00 ng/mL), respectively (Table 2). Endotoxin and glucan concentrations in individual samples were positively correlated (Spearman correlation coefficient $r_s = 0.26$, $p = 0.03$).

On average, glucan concentrations were 4,123% higher (95% CI: 1,408, 11,727%, $p < 0.0001$) in EC cartridges relative to e-liquid samples (Table 3 and Figure 1), though the difference decreased to 318% (95% CI: −10, 1,842%, $p = 0.07$) when adjusted for brand and flavor (Table 3). When adjusted for brand and product type, glucan concentrations were 1,042% (95% CI: 184, 4,489%, $p = 0.001$) and 350% (95% CI: 11, 1,733%, $p = 0.04$) higher in tobacco- and menthol-flavored products than concentrations found in fruit-flavored products, but similar for other-flavored products (112% higher, 95% CI: −45, 710%; $p = 0.3$). Endotoxin concentrations did not show clear differences according to product type or flavor.

Discussion

To our knowledge, this study is the first to identify endotoxin and glucan in EC cartridges and e-liquids sold in the United States. Endotoxin was detected in 17 of 75 (23%) of EC samples, and glucan was detected in 61 of 75 (81%) of EC samples. After adjusting for brand and flavor, the estimated mean glucan concentration in EC cartridges was 3.2 times higher (95% CI: −0.10, 18) than in concentrations found in e-liquids. When adjusted for brand and product type, glucan concentrations in tobacco- and menthol-flavored ECs were 10 times (95% CI: 1.8, 45) and 3.5

Table 2. Characteristics and endotoxin and glucan concentrations of 75 products included in the study sample according to product type, flavor, and brand.

		Endotoxin (EU/mL)[a]					Glucan (ng/mL)[a]					Both[b]
	n (%)	>LOD *n* (%)	GM ± GSD	Median	Min	Max	>LOD *n* (%)	GM ± GSD	Median	Min	Max	<LOD *n* (%)
Overall	75 (100)	17 (23)	0.14 ± 3.18	0.10	0.05	1.64	61 (81)	1.01 ± 18.95	1.06	0.01	1,450.00	13 (17)
By type												
Cartridge	37 (49)	12 (32)	0.18 ± 3.18	0.20	0.05	1.64	37 (100)	6.71 ± 5.42	7.15	0.37	1,450.00	0 (0)
E-liquid	38 (51)	5 (13)	0.11 ± 3.05	0.05	0.05	0.89	24 (63)	0.16 ± 15.27	0.10	0.01	202.80	13 (34)
By flavor												
Tobacco	16 (21)	4 (25)	0.12 ± 2.75	0.08	0.05	0.74	16 (100)	5.13 ± 11.52	9.30	0.03	202.80	0 (0)
Menthol	15 (20)	1 (7)	0.12 ± 3.06	0.05	0.05	0.80	13 (87)	1.88 ± 14.09	5.73	0.01	38.20	2 (13)
Fruit	29 (39)	7 (24)	0.16 ± 3.39	0.10	0.05	0.89	19 (66)	0.20 ± 14.73	0.09	0.01	113.40	9 (31)
Others	15 (20)	5 (33)	0.15 ± 3.59	0.05	0.05	1.64	13 (87)	2.09 ± 18.66	5.40	0.02	1,450.00	2 (13)
By brand												
A	7 (9)	4 (57)	0.37 ± 2.36	0.40	0.10	1.64	7 (100)	20.69 ± 10.84	5.40	3.30	1,450.00	0 (0)
B	4 (5)	0 (0)	0.07 ± 1.49	0.08	0.05	0.10	4 (100)	0.48 ± 2.79	0.63	0.12	1.24	0 (0)
C	19 (25)	4 (21)	0.14 ± 3.22	0.10	0.05	0.89	19 (100)	1.07 ± 11.68	0.45	0.08	36.20	0 (0)
D	2 (3)	0 (0)	0.05 ± 1.00	0.05	0.05	0.05	2 (100)	1.30 ± 1.07	1.30	1.24	1.36	0 (0)
E	2 (3)	1 (50)	0.77 ± 1.06	0.77	0.74	0.80	2 (100)	18.07 ± 1.15	18.15	16.40	19.90	0 (0)
F	7 (9)	2 (29)	0.17 ± 3.48	0.20	0.05	0.80	3 (43)	0.14 ± 38.24	0.02	0.01	202.80	3 (43)
G	6 (8)	1 (17)	0.09 ± 2.36	0.05	0.05	0.33	6 (100)	5.74 ± 1.90	7.03	1.60	9.73	0 (0)
H	6 (8)	4 (67)	0.60 ± 1.73	0.73	0.20	0.83	6 (100)	8.43 ± 2.09	12.35	2.20	13.70	0 (0)
I	18 (24)	1 (6)	0.07 ± 2.38	0.05	0.05	0.80	8 (44)	0.07 ± 10.42	0.03	0.01	113.40	10 (56)
J	4 (5)	0 (0)	0.07 ± 2.00	0.05	0.05	0.20	4 (100)	21.09 ± 1.21	21.75	16.70	25.30	0 (0)

Note: See Table S2 for characteristics and concentrations in each product tested.
[a]Concentrations less than LOD were replaced by LOD/2 when calculating distributions. LODs ranged from 0.1–1.6 EU/mL for endotoxin and from 0.0125–0.2 ng/mL for glucan.
[b]Number (%) of samples with measured concentrations less than LOD for both endotoxin and glucan.

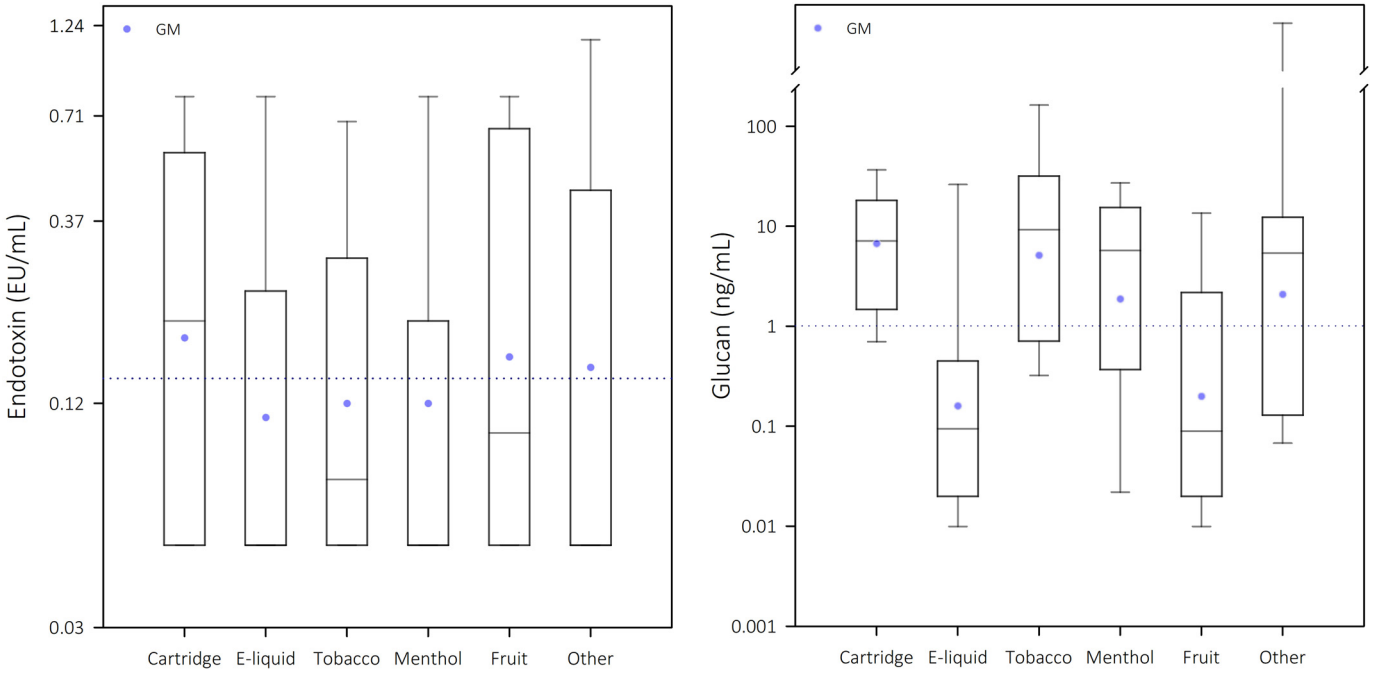

Figure 1. Boxplots showing the median (horizontal line in box), interquartile range (the central rectangle), and the fifth and the 95th percentiles (whiskers above and below the box) of endotoxin and glucan levels by EC type and flavor. Geometric means (GM) by type and flavor are shown as dots. Overall GMs (0.14 EU/mL for endotoxin and 1.01 mg/mL for glucan) are shown as horizontal dotted lines. Median endotoxin concentrations in e-liquid samples, and menthol- and other-flavor samples are not visible as separate lines because they are equal to the concentration at the fifth percentile (0.05 EU/mL).

times (95% CI: 0.11, 17) higher than concentrations in fruit-flavored ECs, respectively. The products included in our analysis were sampled from high-nicotine products sold by popular brands and were not intended to be a representative sample of all EC products sold in the United States (overall, or by brand, type of product, or flavor). Contamination might have occurred at any point during the production of ingredients or finished EC products, and further research is needed to determine whether microbial contaminants that are present in e-liquids prior to aerosolization result in exposures or health risks to users. However, differences in glucan concentrations among the products included in our study sample may provide clues about sources of contamination and suggest that risks might vary by EC type and flavor.

Previous studies have not evaluated endotoxins in EC products, but Hasday et al. reported the first evidence of bioactive LPS (bacterial endotoxin) in cigarette smoke particles and noted higher bioactive LPS in particles collected from mainstream smoke than in particles collected from sidestream smoke samples (Hasday et al. 1999). Larsson et al. reported that the concentration of bacterial endotoxin in airborne particles sampled from a room in which 15 cigarettes were smoked over 7 h was 120 times higher than in indoor air samples collected from the same room in the absence of smoking (Larsson et al. 2004). Although no scientific evidence supports a hypothesis that current observed levels of endotoxin and glucan in ECs raise health concerns, adverse responses of respiratory and immunological systems to exposure to endotoxin and glucan in epidemiologic studies (responses such as reduced lung function; increase in nonatopic asthma, bronchial hyper-responsiveness and peripheral leucocyte count; inflammation; and airflow obstruction) suggest the potential effects on the inhalation exposure route during EC smoking (Carnes et al. 2017; Castellan et al. 1987; Lai et al. 2012, 2015; Maheswaran et al. 2014; Thorn and Rylander 1998; Thorne et al. 2015).

Cartridge ECs contained wicks made of cotton or other fibers (Chun et al. 2017). Endotoxin and glucan are biological contaminants of cotton fibers (Lane and Sewell 2006); thus, contamination of cartridge wicks may be a source of endotoxin

Table 3. Percent differences [95% confidence intervals (CIs)] for endotoxin and glucan levels associated with type and flavor.

	Endotoxin				Glucan			
	Unadjusted models		Adjusted model		Unadjusted models		Adjusted model	
	% Difference	*p*-Value	% Difference	*p*-Value	% Difference	*p*-Value	% Difference	*p*-Value
Type[a]								
Cartridge	66 (−1, 178)	0.06	23 (−42, 159)	0.6	4123 (1408, 11727)	<0.001	318 (−10, 1842)	0.07
E-liquid	Ref		Ref		Ref		Ref	
Flavor[b]								
Tobacco	−26 (−64, 50)	0.4	−40 (−69, 19)	0.1	2421 (391, 12845)	<0.001	1042 (184, 4489)	0.001
Menthol	−27 (−65, 52)	0.4	−39 (−69, 20)	0.2	822 (73, 4800)	0.01	350 (11, 1733)	0.04
Others	−6 (−55, 94)	0.9	−19 (−58, 56)	0.5	928 (93, 5363)	0.01	112 (−45, 710)	0.3
Fruit	Ref		Ref		Ref		Ref	

Note: Sample levels below LOD are substituted by a half the LOD. LODs ranged from 0.1 to 1.6 EU/mL for endotoxin and from 0.0125 to 0.2 ng/mL for glucan. Ref, reference.
[a]Adjusted for flavor and brand (A to J).
[b]Adjusted for type and brand (A to J).

and glucan contamination and might contribute to higher concentrations of glucans in cartridge ECs than in e-liquids. Tobacco-, menthol-, and other-flavored ECs had higher glucan concentrations than concentrations found in fruit-flavored ECs, but differences were attenuated after adjusting for EC type and brand. In contrast, endotoxin concentrations were higher in fruit-flavored products than concentrations found in other products, though differences were not significant. Raw materials used to manufacture flavors might be a source of microbial contamination, but contamination during the manufacture of flavors, other EC components, or finished EC products is also possible.

In 2016, the U.S. FDA issued a final rule to begin regulating ECs as tobacco products under the Family Smoking Prevention and Tobacco Control Act (2009), which defines tobacco products as "any product made or derived from tobacco that is intended for human consumption, including any component, part, or accessory of a tobacco product." We analyzed EC products containing the highest strength of nicotine in each brand and flavor, but we do not know the origin of the nicotine used in the tested EC products, which may be natural nicotine obtained from tobacco leaves or tobacco-free synthetic nicotine. Nicotine derived from tobacco leaves might be a source of endotoxin or glucan contamination, but synthetic nicotine also might be contaminated during manufacturing.

We acknowledge several limitations. We tested only for contamination of samples from cartridges and e-liquids, which may differ from other types of EC products, such as second-generation (pens), third-generation (tanks/MODs), and fourth-generation (pods) devices. We did not test multiple samples of the same product to assess variation among different batches or packages of the same product. In addition, we identified endotoxin and glucan in samples from cartridges and bottles of e-liquids, but we did not evaluate contamination of aerosols inhaled by users. Finally, we analyzed small numbers of products that were selected from popular brands. Future studies should include larger numbers of products, should test products that have been systematically sampled to be representative of all EC products sold in the United States, should perform repeat tests of individual products from different production batches to assess within-product variability, should conduct targeted testing to evaluate specific sources of contamination and variation among different types of EC products, and should measure endotoxin and glucan concentrations in aerosol samples.

In conclusion, our findings indicate that some popular EC brands and flavors may be contaminated with microbial toxins. Additional research is needed to confirm our findings and assess potential exposures and health effects in EC users.

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